

Amendments to the Specification:

Please replace the header “FIGURES” on page 26 with “BRIEF DESCRIPTION OF THE DRAWINGS”.

Please replace on page 26 a paragraph starting with “Figure 1. General Scheme ...” with the following rewritten paragraph:

Figures 1A-1D ~~Figure 1.~~ General scheme of the proposed methods. (A) Schematic drawing of a DNA, allowing conditional exchange of fragment 1 (or fragment A) by fragment 2 (or fragment B), consisting of SSRTS L1, DNA fragment 1, SSRTS L2 in sense orientation, DNA fragment 2, in antisense orientation, SSRTS L1 and SSRTS L2 reversely orientated to the first SSRTS L1 and to the first SSRTS L2, respectively. (B) Intermediate step after recombinase-mediated inversion at SSRTS L1, leading to directly repeated SSRTS L2 (asterisk) flanking DNA fragment 1. This reaction represents an equilibrium with the substrate (A). (C) Intermediate step after recombinase-mediated inversion at SSRTS L2, leading to directly repeated SSRTS L1 (asterisk) flanking DNA fragment 1. This reaction represents an equilibrium with the substrate (A). (D) Final DNA after recombinase-mediated excision of DNA fragment 1 between the directly repeated SSRTS (asterisks). This reaction is not reversible and will shift the equilibrium from the first reaction towards the product (D).

Please replace a bridging paragraph on pages 26-27 with the following rewritten paragraph:

Figures 2A-D ~~Figure 2.~~ Schematic representation of the construct pFlExR and of the expected plasmids after Cre-mediated rearrangement. (A) pFlExR (SEQ ID N.degree. 54) contains, in the following order, the SV40 promoter (broken arrow), a loxP site (open arrowhead), a lox511 site (closed arrowhead), the coding sequence for the enhanced-green fluorescent protein (EGFP) linked to a poly-adenylation signal, the .beta.-galactosidase promoter-less minigene (LacZ) in the antisense orientation, a loxP and a lox511 sites in inverted orientations. The SV40 promoter first drives the expression of EGFP. (B) Intermediate step after Cre-mediated inversion at the loxP sites. (C) Intermediate step after Cre-mediated inversion at the lox511 sites. (D) Final product after Cre-mediated excision between the two

lox511 or the two loxP sites (asterisks). In this plasmid, SV40 promoter now drives beta-galactosidase expression. This reaction is not reversible, as the final plasmid contains single loxP and lox511 sites, which cannot recombine together.

Please replace a bridging paragraph on pages 27-28 with the following rewritten paragraph:

Figures 3A-D ~~Figure 3~~. In vitro Cre recombinase-mediated inversion/excision assay. (A) Schematic drawing of the ploxLacZlox construct used to check for Cre preparation efficiency before (upper panel) and after (lower panel) Cre-mediated recombination. EcoRV restriction sites and location of probes 1 and 2 are indicated. (B) Schematic drawing of pFExR (SEQ ID N.degree. 54) before (upper panel) and after (lower panel, pFExRrec) Cre-mediated recombination. EcoRV and XbaI restriction sites, together with location of probes 1 and 2 are indicated. (C) Evidence for Cre-mediated recombination by Southern blot analysis of plasmids digested with EcoRV and XbaI using probe 1. Lane 1 and 2, loxP-flanked LacZ plasmid (ploxLacZlox); lane 3 and 4, pFExR; lane 5 and 6, pFExRrec (inverted/excised pFExR, see Materials and Methods). A crude Cre preparation was added in reactions illustrated in lanes 2, 4 and 6, whereas a heat-inactivated Cre preparation was added in reactions shown in lanes 1, 3 and 5. (D) Evidence for Cre-mediated recombination probing the same Southern blot as in (C) using probe 2 (for details see Materials and Methods). Note that the excised lacZlox fragment (3.7 kb), which does not contain plasmid sequences, was lost during amplification in bacteria. Open arrowhead, loxP site; closed arrowhead, lox511 site.

Please replace on page 28 a paragraph starting with “Figure 4. *In vivo* ...” with the following rewritten paragraph:

Figures 4A-H ~~Figure 4~~. In vivo Cre recombinase-mediated inversion/excision assay. COS-1 cells were transiently transfected with either pFExR in absence (A and C) or in presence (B and D) of five fold excess pSG5-Cre, and transfected with pFExRrec in absence (E and G) or in presence (F and H) of excess pSG5-Cre. Detection of EGFP was examined by fluorescence microscopy (A, B, E and F) and LacZ expression was assessed by light microscopy after X-

Gal staining (C, D, G and H). Note that the background blue staining in FIG. 3C most probably reflects a low level of transcription of the beta-galactosidase minigene initiated from the non-coding strand of the pFLEXR.

Please replace a bridging paragraph on pages 28-29 with the following rewritten paragraph:

Figures 5A-D. ~~Figure 5:~~ Generation of a conditional RAR.gamma. allele by homologous recombination. (A) Schematic drawing of the RAR.gamma. locus. Exons 7 to 14 are shown as solid boxes. As indicated, E7 is specific for RAR.gamma..sup.2, while E8 to E14 are common to all isoforms. The promoter (P2) is indicated by a broken arrow. 5' and 3' untranslated regions are shown as white boxes. Exon 8, whose splice acceptor is shown as waved lines, was chosen for the conditional disruption of RAR.gamma.. (B) Structure of the targeting vector (p.gamma.6.0Flex.beta.-Gal) (SEQ ID N.degree. 55). (C) Structure of the recombinant allele following homologous recombination. (D) Structure of the recombinant allele after FLP-mediated removal of the selection cassette.

Please replace on page 29 a paragraph starting with “Figure 6: Expected Structures ...” with the following rewritten paragraph:

Figures 6A-D. ~~Figure 6:~~ Expected structures of the RAR.gamma. locus after Cre mediated recombination. (A) Structure of the modified RAR.gamma. locus, after FLP-mediated removal of the selection cassette. Dotted lines represent the expected splicing of the primary transcript. (B) Transient structure of RAR.gamma. locus of A after Cre-mediated inversion of the DNA fragment flanked by loxP sites. The asterisk points to the direct repeat of lox511 sites. (C) Transient structure of RAR.gamma. locus after Cre-mediated inversion of the DNA fragment flanked by lox511 sites. The asterisk points to the direct repeat of loxP sites. (D) Structure of RAR.gamma. locus after Cre-mediated excision at the repeated lox sites (asterisks in B and C). Dotted lines represent the expected splicing of the primary transcript.

Please replace on pages 29-31 a paragraph starting with “Figure 7. Possible ...” with the following rewritten paragraph:

Figures 7A-E. ~~Figure 7.~~ Possible applications of the present invention. (A) Conditional knockout linked to simultaneous activation of a reporter. The scheme represents a conditional allele expressing the wild type protein (left side); upon Cre-mediated rearrangement (right side), exon 2 is removed and replaced by the reporter gene and its polyadenylation signal. Thus, replacement of the normal gene product by the reporter protein renders possible the direct identification of individual cells that underwent recombination (i.e. gene knockout). (B) Cassette exchange. The scheme represents a locus after Cre-mediated inversion/excision (left side). A further Cre-mediated rearrangement in the presence of a circular DNA containing a loxP-Cre-lox511 cassette (right side) leads to the exchange between the reporter and Cre genes. (C) Conditional rescue. The scheme represents a knock-in reporter allele (left side). After Cre-mediated rearrangement (right side), the reporter cassette is removed together with its polyadenylation signal, while the wild type exon is restored in the sense orientation. (D) Conditional point mutation. The scheme represents a conditional allele expressing the wild type protein (left side). Upon Cre-mediated rearrangement (right side), exon 2 is removed and replaced by mutated exon 2 (E2m), giving rise to the synthesis of a mutated protein. (E) Conditional gene replacement. The scheme represents a conditional allele expressing the wild type protein (left side). After Cre-mediated rearrangement (right side), exon 2 is removed and replaced by a cassette containing an internal ribosomal entry site (IRES) followed by a chosen cDNA and a polyadenylation signal. Synthesis of the wild type protein is abrogated, whereas the introduced cDNA is now expressed. Dotted lines represent the expected splicing of the primary transcript, and E1 to E3 stands for exons. Open and closed arrowheads represent loxP and lox511, respectively.

Please replace on page 31 a paragraph starting with “Figure 8: Description ...” with the following rewritten paragraph:

Figures 8A-D. ~~Figure 8.~~ Description of the construct pJMG and the expected variants after Cre-mediated rearrangement. (A) Schematic drawing of pJMG which contains, in the following order, an FRT site (closed flag), a loxP site (open arrowhead), a lox511 site (closed arrowhead), a DNA cassette consisting of the rabbit .beta.-globin intron splice acceptor site (SA), an IRES sequence linked to the promoter-less nls-.beta.-galactosidase mini gene (LacZ)

and a loxP site in antisense orientation, a PGK promoter (broken arrow) driving expression of the neomycin phosphotransferase coding sequence (Neo) linked to the OBS sequence and a synthetic splice donor (SD), a lox511 site and a mutated FRT site (FRTm; open flag) in antisense orientation. (B) Intermediate step after Cre-mediated inversion at the loxP sites. (C) Intermediate step after Cre-mediated inversion at the lox511 sites. (D) Final product after Cre-mediated excision between the two lox511 or the two loxP sites (asterisks), removing the PGK Neo Cassette. This reaction is not reversible, as the final plasmid contains single loxP and lox511 sites, which cannot recombine together.

Please replace a bridging paragraph on pages 31-32 with the following rewritten paragraph:

Figures 9A-C. ~~Figure 9:~~ In vitro Cre recombinase-mediated inversion/excision assay on plasmid pJMG (SEQ ID N.degree. 56). (A) Schematic drawing of pJMG (upper panel), the intermediate construct pJMG-f (middle panel) and the final construct pJMG-fx (lower panel). HindIII restriction sites, together with the location of the probe are indicated. (B) Evidence for Cre-mediated recombination assessed by ethidium bromide stained agarose gel analysis of HindIII digested plasmids. Lane 1 and 2, loxP-flanked LacZ plasmid (ploxLacZlox); lane 3 and 4, pJMG; lane 5 and 6, pJMG-f (inverted pJMG, see Materials and Methods); lane 7 and 8, pJMG-fx (inverted and excised pJMG, see Materials and Methods). A Cre preparation was added in the reactions illustrated in lanes 2, 4, 6 and 8, whereas a heat-inactivated Cre preparation was added in the reactions shown in lanes 1, 3, 5 and 7. The sizes of the expected HindIII fragments are indicated on the right. (C) Evidence for Cre-mediated recombination assessed by Southern blot using a probe recognizing the rabbit beta-globin splice acceptor site (for details see Materials and Methods) Note that this probe does not hybridise to the ploxLacZlox. Open arrowhead, loxP site; closed arrowhead, lox511 site; closed flag, FRT site; open flag, FRTm site, SD, synthetic splice donor.

Please replace a bridging paragraph on pages 32-33 with the following rewritten paragraph:

Figures 10A-D. ~~Figure 10:~~ Scheme of the gene trap strategy. Upon insertion of the pJMG vector into an intron of a transcribed locus, transcription of the trapped gene should not be affected (Trapped allele, WT). The PGK promoter drives the expression of the NEO cassette linked to the 3' part of the trapped gene that provides the poly-A signal necessary to produce a stable mRNA. The cell is thus resistant to G418 selection (NeoR), whereas the LacZ gene is silent (LacZ⁰). Upon Cre-mediated recombination, the Neo gene is removed and LacZ is inverted. The cell becomes sensitive to G418 selection (NeoS), whereas the LacZ gene is expressed under the control of the trapped promoter (LacZ⁺). Dotted lines represent the expected splicing of the primary transcript; E1 and E2 stands for exons 1 and 2; SA indicates rabbit .beta.-globin splice acceptor site; IRES stands for internal ribosomal entry site. Open and closed arrowheads represent loxP and lox511 sites, respectively. Closed and open flags represent FRT and mutated FRT sites, respectively, SD, synthetic splice donor.

Please replace on page 33 a paragraph starting with “Figure 11: *In vivo*...” with the following rewritten paragraph:

Figures 11A-I. ~~Figure 11:~~ In vivo assay to test for functionality of the splice acceptor-IRES LacZ cassette and/or the polyA trap based method in F9 cells. F9 cells were stably transfected with the NotI fragment of pJMG-f, selected against G418 for 12 days and subjected to X-gal staining. As expected, neomycin resistant clones were obtained, out of which some of them expressed lacZ. Panels A-1 depict individual clones showing varying degrees of activity.

Please replace on pages 33-35 a paragraph starting with “Figure 12: Possible applications...” with the following rewritten paragraph:

Figures 12A-I. ~~Figure 12:~~ Possible applications using recombinase mediated cassette exchange. (A) The scheme represents a trapped genetic locus after Cre-mediated inversion/excision (left side). The LacZ reporter is expressed under the control of the trapped gene promoter. A further Cre- or FLP-mediated rearrangement, in the presence of a circular DNA containing a Cre cassette flanked with appropriate recombinases specific target sites,

leads to the exchange between the reporter and Cre (right side). Then Cre is expressed under the control of the trapped gene promoter. (B) The scheme represents a trapped genetic locus after Cre-mediated inversion/excision (left side). A further FLP-mediated recombination in the presence of a circular DNA containing a cDNA cassette flanked with a FRT sequence on its 5' side and of a FRTm sequence on its 3' side leads to the exchange between the reporter and cDNA (right side). As the cDNA is itself flanked by two pairs of loxP and lox511 sites, expression of the cDNA depends on the presence of Cre (conditional allele upon Cre-mediated recombination). (C) The scheme represents a trapped genetic locus after Cre-mediated inversion/excision (left side). A further Cre-mediated recombination in the presence of a circular DNA containing a cDNA cassette flanked with a loxP sequence on its 5' side and of a lox511 sequence on its 3' side leads to the exchange between the reporter and cDNA (right side). As the cDNA is itself flanked by a FRT sequence on its 5' side and of a FRTm sequence on its 3' side, expression of the cDNA depends on the presence of FLP (conditional allele upon FLP-mediated recombination). Dotted lines represent the expected splicing of the primary transcript; E1 and E2 stand for exons 1 and 2; SA indicates rabbit .beta.-globin splice acceptor site; IRES stands for internal ribosomal entry site. Open and closed arrowheads represent loxP and lox511 sites, respectively. Closed and open flags represent FRT and FRTm sites, respectively.